Australian Journal of Basic and Applied Sciences, 6(7): 497-509, 2012
ISSN 1991-8178

Effect of Lead Toxicity on Cytogenisity, Biochemical Constituents and Tissue Residue with Protective Role of Activated Charcoal and Casein in Male Rats

Hanan, A. Azoz and Riham, M. Raafat

Dept. of Biochemistry, Animal Health Research Institute, Dokki, Egypt.

Abstract: Lead is a common industrial and environmental pollutant. Prolonged exposure of a sub-lethal dose to this toxicant is associated with oxidative stress, damage of DNA and considered to be a risk factor for kidney, liver added to many disorders. This study was carried out to investigate the most toxic effects of lead with trial to diminish this toxicity by supplementation of casein or activated charcoal. Therefore, forty mature male albino rats were used , they divided into four equal groups, 10 rats of each. Group 1 was considered as control. Rats of other groups (2, 3 and 4) were supplemented lead acetate at a dose of 0.5 g/100 ml drinking water for 2 months. Gr 3 is given charcoal at a concentration of 0.05g ration, while in Gr 4 casein is mixed with ration at a concentration of 20 g/100 g ration. Group 2 revealed significant increases in serum AST, ALT, ALP, LDH, GGT, TB, TC, LDL and CAT levels and decreased total protein, HLDL, VLDL, trace mineral salts, SOD and GSH- px. The results of haematological study evoked a significant decrease in the red blood cells (RBCs) count, blood haemoglobin (Hb) concentration and packed cell volume (PCV). Cytogenetic study resulted a significant increase in the percent of multinucleated polychromatic erythrocytes (MPCE) as well as significant increase in the polychromatic- erythrocytes (PCE) and normochromatic erythrocyte (NCE) ratio (PCER/NCE) Lead concentration in level serum and different tissues showed lead accumulation in serum, kidney, liver, muscle, intestine and spleen. Supplementation of charcoal and casein induce protective effects against lead toxicity in most of above parameter studies, and casein showed higher efficacy in the protection of lead- induced toxicity than charcoal.

Key words: lead toxicity, cytogenisity, biochemical constituents, tissue residue

INTRODUCTION

Lead (Pb) is a heavy soft metal occurs in nature as oxide or salts, it is one of the most hazardous and cumulative environmental pollutants (Milnes *et al*., 2006). Lead is one of the most frequently reported causes of poisoning in farm animals especially in cattle (Baars *et al*., 1988).Sources of lead poisoing in animals are due to suckling lead paint or lead toy and drinking water contaminated with petroleum industries, The major source of soil contamination is waste, combustion of leaded gasoline and use lead arsenate pesticide (National Academy of Science, 1972).

Effect of chronic lead poisoning are the disorders in gastrointestinal and haemopoietic system and muscular weakness leading to paralysis (Aly *et al*., 1993). Lead is distributed in tissues depends on administration rout and chemical form (Blaxter, 1950 and National Academy of Science, 1972) . Most of the lead entering the systemic circulation by ingestion invades the reticuloendothelial system represented by bone marrow, spleen and liver. In contrast, that entering the gut wall goes to bone and kidney (Blaxter, 1950).

Lead ions damage chromosome structure and induce increase in chromosome aberration in mammalian species (Paton, 1973; Dhir *et al*., 1990 and Hatwig *et al*., 1990). Lead nitrate induced micronuclei in bone marrow cells of mice, micronuclei are chromatid / chromosome fragments that are left behind after expulsion of the main nucleus during maturation of erythroblasts to erythrocytes in the bone marrow. These represent the consequence of DNA damage caused by externally administered substance (Frangenberg, 1986).

The concentration of lead residues in tissues of farm animals depend upon the rout of entering and period of exposure to the environmental pollutants (air, water and plants) where inhalation of polluted dust on fumes was more dangerous than oral ingestion of polluted water and plants for long period. Most orally ingested lead is deposited in the skeleton (National Academy of Science, 1972). Initially, lead deposited in bone until a possible threshold is reached then it deposited in other tissues especially the kidneys. The lead particles which ingested or inhalated pass to the blood stream and 82.0% was excreted in faeces and urine. Only 0.5% was excreted with the milk and the rest 17.95% remains and restored in tissues and body organs (Baars *et al*., 1988). Frangenberg (1986) found that administration of very high dose of lead to animals resulted in highest lead accumulation in the kidney then liver, bone marrow, brain and finally in the heart muscles. Whereas, chronic oral administration of low doses of lead resulted in accumulation particularly in bone marrow, kidney and skeleton muscle in most animal species

Casein is a protein fraction from bovine milk and is responsible for binding to many ligands (Srinivas *et al*., 2007).

Corresponding Author: Hanan, A. Azoz, Dept. of Biochemistry, Animal Health Research Institute, Dokki, Egypt
E mail: hanan.a.azoz@hotmail.com

A relationship between susceptibility to lead toxicity and dietary content of protein (such as casein) and certain amino acids has been observed by Gontzeer *et al.* (1964).

Activated charcoal (AC) denotes a material which has an exceptionally high surface area and includes a large amount of microporousity. It is cheap, harmless and easily used. Ac is produced from the controlled burning of wood or bone, which is then subjected to the action of an oxidizing gas such as steam or air at elevated temperature (Ellenhorn, 1997). This process enhances the adsorptive power of charcoal by developing an extensive network of fine pores. Ademoyero and Dalvi (1983) and Jindal *et al.* (1994) used Ac to reduce the toxic effects of aflatoxin in chicken. Azab and Peterson (1999) removed Cd from waste water by using AC.

The purpose of the present study was to investigate the hazardous effect of lead toxicity and trials to diminish these draitic effects in the body by using non –specific chelating agents as casein milk or activated charcoal.

MATERIALS AND METHODS

Materials:

[1] Chemicals:

(a) Lead (Pb): lead acetate trihydrate ($C_4H_6O_4Pb_3H_{20}$) of molecular weight 379.33 was provided by Riedel Dehaen, Hannover, Germany. Each one gram of pb is found in 1.8307 g of finely powders of lead acetate (Fatma, 1992).

(b) Activated charcoal (AC): It was purchased from Arab Drug Company in the form of tablets containing 100 mg of AC which was grind and mixed with the ration.

(c) Casein: It is a white amorphous powder without odour or taste very sparingly soluble in water and in nonpolar organic solvent and soluble in aqueous solution of alkalis of molecular weight 23.600 (Ribadeaudumas, 1972). Casein (pure form) was obtained from El Gomhorya Company, Egypt.

[2] Animals:

Forty mature male albino rats of wister strain, weighing 140 – 160 g were used. The rats were purchased from the National research Center and were allowed to acclimate to their new environment for 7 days prior to initiation of the experiment. Water and food were provided adlibitum.

Methods:

[I] Experimental design: Forty male rats were divided into 4 groups (10 rate / group). The experimental design was shown in table (1)

Table 1:

Group	Supplemental material	Dose	Route	Period of experiment
Group(1) Control	Distilled water			2 months
Group (2)	Lead acetate	0.5g/100ml dist. water	Via drinking water	
Group (3)	Lead acetate +activated charcoal	0.5g/100ml dist.water 0.05g / 100 gm ration	Via drink H_2O + food intake	
Group (4)	Lead + casein	0.5g / 100 ml dist. water 20g/100gm ration	Via drinking water + food intake	

N.B : - lead acetate (0.5g / 100 ml dist. water) according to Jin, *et al.* (2008)
- Activated charcoal (0.05g / 100 g ration according to Cupic *et al.* (2003).
- Casein (20 g / 100 g ration) according to Blanusa, *et al.* (1989).

[II] Sampling

1) Blood Samples:

At the end of the experiment blood samples were collected from eye canthus. Each sample was divided into two portions.

a- The first one mixed with anticoagulant dipotassuim ethylene diamine acetic acid (EDTA) for studying the hemogram including values of hemoglobin (Hb), packed cell volume (PCV) and total erythrocytic count (TEC) by conventional methods.

b- The second portion was allowed to clot then centrifugated at 3000 rpm for 20 min. for serum separation to determine some biochemical parameters.

2) Tissues Samples:

Animals were sacrificed, and tissue samples from (liver, kidney, muscle, spleen and intestine) were obtained from all animals in all groups for determination of lead residues.

[III]: Biochemical Analysis:

Serum total protein according to the method of Dumas *et al*. (1971).

Total bilirubin accord. to Walter and Gerade (1970), serum cholesterol according to method of Richmond (1973), HDL (high density lipoprotein) according to method of Assmann (1979).

LDL low density lipoportein according to Bachorik and Ross (1995).

LDH Lactate dehydrogenase according to method of Szasz *et al*. (1974).

Serum HDL and LDL (low density lipoprotein) + VLDL (very low density lipoprotein) were separated by the heparin manganese precipitation method according to Warnick and Albers (1978).

- Triglyceride spectrophotometrically by glycerol oxidase method adopted by Wahlrfeld (1974).

GGT according to method of William (1980).

AST, the activities serum aspartate aminotransferase and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (1957). Alkaline phosphate (ALP) was determined according to Dumas *et al*. (1971).

Colourimetric determination was done for calcium (Glinder and king, 1972), Zinc (Makino *et al*., 1982) iron (Ariss *et al*., 1981) and copper were determined by atomic absorption according to Linda *et al*. (1988). Serum selenium was determined by electrothermal atomic absorption spectrometry with Zeeman background correction using a palladium chloride chemical modifier (Ghayour-Mobarhan *et al.,* 2005) Typical between batch precision (CVs) was 3.7%

[IV]: Antioxidants Study:

Determination of antioxidant enzymes levels in serum of rats in different experimental groups:

1- Superoxide Dismutase (SOD)

SOD activity was determined by the epinephrine method according to Mirsa and Fridovich (1972).

2- Estimation of catalase activity was measured by the previous method Beuter (1982).

3- Reduced glutathione (GSH) content was estimated according to Beutler, and Gelbart . (1969).

4- Estimation of glutathione peroxides (GSH-PX) was assayed by following the oxidations of NADPH at 340 nm with t. butyl – hydroperoxid according to Tamura *et al*. (1982).

[IV] The Hemogram of Animals:

including values of hemoglobin (Hb), packed cell volume (PCV) and total erythrocytic count were determined according to Bernard *et al*., (2000).

[VI]: Cytogenic Study:

The micronucleus test was performed to detect chromosomal damage associated with the treatment following the protocol established by Salamon *et al*. (1980). Bone marrow cells of each rat of the all groups were extruded with a pin into a clean dry glass slide and homogenized with two drops of fetal calf serum. Cells were smeared on the slide, air dried fixed in absolute methanol and stained with Geimsa stain in phosphate buffer pH 6.8. The polychromatic erythrocytes (PCE 1000 per animal) were screened for micronuclei and changes in the mitotic activity (Hart and engberg-Pederson, 1983 and AL.Bekari *et al*., 1991) were assessed on the basis of ratio polychromatic to normochromatic erythrocytes (PCE / NCE ratio).

[VII]: Determination of Lead Residue:

measurement of lead residues in liver, Kidney, muscle, spleen intestine and serum were determined by Flame atomic absorption spectrometry (AAS). The organ samples (1 gm) were prepared using wet digestion method with perchloric acid and nitric acids ratio (5:1) according to Graig and Wayne (1984).

[VIII]: Statistical Analysis:

The obtained data were statistically analysed using F. Test – ANOVA test acc. to Snedecor and Cochran (1980)

RESULTS AND DISCUSSION

Lead is a pervasive environmental pollutant that accumulates in almost all tissues (Doyle and Younger, 1984) and with no beneficial biological role (Valverde *et al*., 2002). Our study is concerned with investigation to the toxic effects of lead exposure on liver and kidney, some essential body's elements and bone marrow. In addition, determination of its probable residues in serum and some tissues. Finally, comparison of charcoal and casein supplementation in prevention or decreasing the lead toxicity which is the main target of our study.

Our data showed that lead exposure in (Gr 2) caused significant increase in the activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Gamma glutamyltransferase (GGT), lactate dehydrogenase (LDH) and total bilirubin, while the level of total protein was

decreased. Similar results were reported by Shalan, *et al*. (2005), Herman *et al*. (2009), Khan, *et al*. (2008), Moussa and Bashandy. (2008), Mehana, *et al*. (2010), Lynda Allonche, *et al*. (2011) and Nabil *et al*. (2012).

Increasing of the serum activities of AST and ALT was most likely a consequence of the hepatotoxic effect of lead i.e the occurrence of toxic hepatitis. The lead entering the body by ingestion is delivered to the liver through the portal blood circulation and smaller part of the lead "break the liver barrier" and enters the body circulation. The accumulated lead in the liver can act by directly damaging the hepatocytes primarily by destroying the permeability of the cell membrane, with resultant release of cellular enzymes leading to increase their serum values (Todorvic, *et al*., 2005). Durgut, *et al*. (2008) found that, the increasing serum activities of AST and ALT are associated with liver damage and or cardiac or skeletal muscle damage. Furthermore Nabil *et al*. (2012) reported that the high plasma AST and ALT activities are accompanied by high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue.

In our study, lead (supplemention) significantly increase serum ALP activity which causes a higher level of introhepatic cholestase (Todorovic, *et al*., 2005). Increasing in this enzyme can be indicator of both liver damage and bone damage which are associated to the increased activity of osteoblasts (Maykic-singh, 1993).

Alkaline phosphatase found in the walls of the intra and extra – biliary ducts. Elevation of this enzyme may indicate an injury to the biliary cells (Fauci *et al*., 2010). In addition, alkaline phosphatase can be considered as markers of the possible neurotoxicity of lead. Intoxication with lead was associated with alterations which cause renal toxicity and damage (Antinio *et al*., 2003).

In our results lead exposure in Gr2 showed significant increase in LDH which may be due to alteration in redox status as indicated by a decrease in glutathione levels, an increase in lipid peroxidation end product – 4 – hydroxynonenal levels which may be produced by damage in RBCs membrane and increased LDH in plasma (Seddik *et al*., 2010).

Moreover our data revealed that supplementation of lead acetate in (Gr 2) induced a significant decrease in the values of serum total proteins (hypo proteinemia) of our result in accordance with Nabil *et al*. (2012). The decrease in serum total protein may be due to both hepatic and renal damage induced by lead (Ahmed and Shalaby, 1999), or may be due to binding of lead to plasmatic proteins, where it causes alteration in a high number of enzymes and can also disturb protein synthesis in hepatocytes (Georing, 1993). Moreover, the decreasing of serum total protein values may be attributed to a decrease in hepatic DNA and RNA induced by lead intoxication or due to decreased utilization of free amino acids for protein synthesis (Shalan *et al*., 2005 and Moussa and Bashandy 2008).

The DNA damage by lead may be due to depletion of antioxidant enzymes (Salma and Kshama, 2005).

The increase of plasma bilirubin values in our study may be due to the induction of heme oxygenase. The catabolism of heme oxygenase is an enzyme which can convert heme to bilirubin. The bilirubin that formed in different tissues is transported to liver as a complex with serum bilirubin. Bilirubin is conjugated with glucuronide in the liver but under the effect of lead toxicity, this conjugation of bilirubin will become inactive. Bilirubin has a protective role against oxidative damage of cell membrane induced by metal (Noriega, *et al*., 2003).

Data of table (3) showed that lead supplementation in Gr 2 had a significant increase of total cholesterol, low density lipoprotein (LDL) and triglyceride, while high density lipoprotein (HDL), very low density lipoprotein (VLDL) were significantly decreased as compared with control group (Gr I) and others groups (Gr3 and Gr 4).

Our findings were supported by studies recorded by Ademuyiwa *et al.* (2005) ; Estela Kristal Boneh *et al.* (2005) ; Hami *et al.* (2006) and Hamadouche *et al*. (2009).

The association between lead exposure and high serum lipid levels is biologically plausible and could be due to either increased synthesis or decreased removal of lipoproteins. Decreased removal may occur as a result of the alteration of cell – surface receptors for lipoprotein (Tarugi , *et al.,* 1982) or as a result of the inhibition of hepatic lipoprotein lipase activity (Chajet ,*et al.,* 1989).

Furthermore, lead has been shown to depress the activity of cytochrome P – 450 (Alvares, *et al* 1975), this can limit the biosynthesis of the bile acids, which is the significant route for elimination of cholesterol from the body. The increasing level of LDL and decreasing in HDL in our study are major cardiovascular risk factors (Dominiczalk, *et al.,* 2000).

Serum Levels of Some Essential Elements:

The rats of Gr2 that exposed to lead acetate at concentration of 0.5 g/100ml drinking water for 2 months showed a significant drop in the serum concentration of copper, zinc, iron, calcium and selenium as compared to control group (Gr 1) and rest groups (Gr 3 and Gr 4) Table (4). Our Data were in accordance with previous results recorded by Spomerika, *et al.* (2007) and Jin *et al. (*2008).

The decrease of zinc concentration after lead exposure may due to the imbalance metabolism produced by impairing zinc status in zinc- dependent enzyme which is necessary for many metabolic processes (Nabil, 2012).

Jin *et al.* (2008) was explained the decreased concentration of zinc and iron by the presence of protein with high binding affinity for lead in red blood cells. For example ALAD (amino levulinic acid dehydrogenase) contains four reactive cysteines that can bind Pb and two different types of zinc- binding sites (Jaffe *et al.,* 2000). In addition the binding of Pb is about 20 times tighter than for zinc (Simons, 1995). The results indicate that lead in blood in much similar to glutathione- Pb, therefore, Pb can replace some of the zinc (Jaffe *et al.,* 2001). Whereupon, it forms structure as the trigonal pyramidal Pb (11) – S_3 or Pb (11) – S_{5-8}. This may cause a decrease in zinc concentrations in the blood. In addition, Ferrochelatase which catalyzes the insertion of iron into protoporphyrin IX is sensitive to lead (Lubran, 1980 and Ahmed and Siddiqui, 2007). Lead induced inhibition of this enzyme leads to an increase in the concentration of zinc proporphyrin (ZPP) which is excreted from the body in the urine. This may also cause a decrease of the zinc and iron concentration.

zinc and copper are essential components of antioxidant enzymes of the body that play an important role in the prevention of free radical – induced damage to tissues (Evans and Halliwel, 2001), in addition zinc protects the peroxidation of membrane lipids, possibly by displacing bound transition metal ions (Bettiger *et al.,* 1980).

Patora and Swarup (2004) recorded that administration of lead significantly decreased zinc and copper concentration in cardiac tissue of calves where it leads to reduce the absorption of micronutrients from gastro-intestinal tract, besides interaction of lead and trace mineral at tissue level.

Table (4) showed pronounce drop in iron level of serum rats exposed to lead (Gr 2) in comparison with control group (Gr 1) and other groups (Gr 3, Gr 4). This result is in accordance with that reported by Jin, *et al.* (2008). The decrease of iron may be due to reduce the activity of ferrochelatase which inhibits the iron insert into proto porphyrin IX, thereby decreasing the iron concentration in the blood. This indicates that lead toxicity inhibits the heme biosynthetic pathway, with the decrease in the hemoglobin level in the bloods.

On the same line, Hande and Nuran (2000) found that lead reduced the absorption of iron from the gastrointestinal tract and inhibited the heme biosynthesis. Moreover, Anuradha (2007) said that transferrin (TF) is the major iron- transport protein in serum and other biological fluids capable of transporting various metals. Lead inhibited TF endocytosis and iron transport across the cell membrane of reticulocytes. This study concides potential effect of lead in the hepatic system and a possible interference in iron metabolism.

In the present study, the concentration of selenium significantly decreased in Gr 2 as compared to others groups. Selenium is well known required for the activity of metallo-enzyme glutathione peroxidase (GSH-PX) which plays a key role in recycling glutathione and is effective in reducing free radicals (Ryan and Aldoori 2005). The rate of free radical production increases with decreasing selenium concentration. This indicates that lead toxicity increase the rate of free radical production where selenium levels is low (Todd *et al.,* 1996). Lead toxicity causes increased production of reactive oxygen species (Ros) and then accelerates production of free radical. The radicals produced decrease in cell membrane fluidity, and increase the rate of erythrocyte hemolysis lead exposure reduces selenium level in the blood by forming highly bonded selenium-lead complexe, which is excreted from the body (Flora *et al*., 1982).

Asa ,*et al.* (1989) found that exposed lead workers had lower plasma levels of selenium, Pb interferes with the metabolism of certain essential elements including copper, zinc and selenium by affecting their absorption, distribution and bioavailability in the body; and can inhibite DNA protein.

Our results in table 4 showed a significant hypocalcaemia in acomparisan with other rest groups .These were similar to results recorded by Winston, *et al.* (1991) and Anetor *et al.* (2005). Hypocalcaemia reflects perturbation of calcium metabolism.

Lead interferes with calcium in several metabolic pathway leads to decrease in calcium level. On the other hand Missoun *et al.* (2010) recorded that of calcium and phosphorus increase in serum of rats administered with lead acetate in drinking water for 8 week. This may be due to impairment of renal function or inhibitory action of lead on cation transport in tissues of rats. The most widely accepted reasone of hypocalcaemia due to lead toxicity is the interference of lead with the final metabolism of vitamin D to the active metabolite, calcitriol (1, 25 – DHCC), a hormone required for adequate calcium absorption (Anetor, 2002).

Activity of Antioxidant Enzymes in Blood:

Levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), catalase (CAT) and reduced glutathione (GSH) were significantly decreased in lead acetate treated rats (Gr 2) as compared to control group (Gr 1) and other groups (Gr 3, Gr 4) table (5). This results similar to results recorded by Gurer *et al.,* (1998), Kulikowska and Moniuszko (2001), Sujatha, *et al.* (2011) and Veena, *et al.*, (2011).

On the other hand, Adebayo *et al.*, (2009) reported that catalase, lipid peroxidation and reduced glutathione reductase significantly increased in blood of lead treated rats but superoxide dismutase not effected thus in acute lead toxicity.

The decrease in SOD activity in rats intoxicated with lead may be due to the decrease in copper concentration in the serum. Copper shows a dualistic character in its actions. The free (Cu^{++}) ions copper are a strong peroxidative factor. On the other hand, this metal is a component of important enzymes such as SOD and ceruloplasmin, which protect against perioxidative processes.

Farmand *et al*., (2005), Alghanzd, *et al.*, (2008) and Sujatha, *et al.,* (2011) mentioned that the change of SOD, GPX and CAT could lead to generate reactive oxygen species (ROS) or by reducing the antioxidant cell defense systems by depleting glutathione or by inhibiting sulfhydryl dependent enzymes or by interfering with some essential metals (copper) needed for antioxidant enzyme activities and by increasing cell susceptibility to oxidative attack by altering the membrane integrity and faulty acid composition. Moreover, Upasani and Balara man (2001) and Patrick (2006) recorded that release reactive oxygen species (ROS) inhibits sulfhydril antioxidant production inhibit enzyme reactions cause nucleic acids damage, inhibit DNA repair, and initiate lipid peroxidation in cellular membranes. Lead toxicity leads to free generation of reactive oxygen species (ROC), including hydro peroxides, singlet oxygen, and hydrogen peroxide and the direct depletion of antioxidant reserves. The enzyme responsible for the decomposition of lipid peroxides is GSH-PX. which protects cellular membranes from peroxidative damage.

Furthermore, Hand and Nuran (2000) found that the antioxidant enzymes depend on various essential trace elements (such as Selenium, copper, zinc) for proper molecular structure and enzymatic activity they are potential targets for lead toxicity.

Lead Residues in Serum and Some Tissues:

Data in table (6) showed that lead accumulated preferentially in the kidneys, liver, muscle, intestine and less in spleen and serum. Lead concentrations in the serum and tissues were significantly higher in Gr 2 than control (Gr 1) and other groups (Gr 3, Gr 4).

These results in accordance with Durgut, *et al.*,(2008) and Fahim, *et al.*,(2009). Previous studies recorded that the higher concentration of lead in tissues were associated with oxidative damage of DNA , protein and lipids leading to lead toxicity (Patora and Swarup, 2004).

Neathery and Miller (1975) and Bogden, *et al.*, (1995) reported that lead distribution in tissues depends on; administration route and its chemical form. Most of the lead enters the systemic circulation by invading the reticuloendothelial system represented by bone marrow, spleen, and liver. They found that oral dosing of lead goes to skeleton until a possible threshold is reached then it is deposited in other tissues especially the kidney and liver.

Increased lead concentration in kidneys may be due to its secretion by glomerular filtration and elevated lead nephropathy which is seen as intranuclear inclusion bodies in the proximal tubules of lead poisoned animal (Milton, *et al.*, 1982).

Haematological and Cytogenicity Studies:

Our results showed that oral administration of lead acetate at a dose of 0.5 g/100 ml in drinking water for 2 months (Gr 2) induced a significant decrease in haemoglobin (Hb), total erythrocytic count and packed cell volume as compared with control and other groups. Our results were in agreement with Durgut *et al*. (2008); Nabil *et al*. (2012) and Hand and Nuran (2000).

Occupational exposure to high levels of lead is known to pose health risks including effects on the blood (anaemia) (De Silva, 1981).

The drop in Hb value was confirmed, the decreases in RBCs which may be attributed to the toxicity of lead acetate. It is in agreement with the elevation of plasma bilirubin level which could be due to the induction of heme oxygenase (Nabil, 2012).

Anuradha (2007) found that lead induced anaemia, results from shortening of erythrocyte life span and an inhibition of haemoglobin synthesis. Lead acts on heme synthesis via its inhibitory effect of ALA dehydrogenase (ALAD), the enzyme involved in the final step of heme synthetic pathway.

In the present study after examination of bone marrow cells a significant increase in percentage of micronucleated polychromatic erythrocyte (MPCE) and PCE/NCE ratio in group 2 compared with group 1 (control). Our results were in accordance with previous results recorded by Alghazal *et al*. (2008); Nashwa et al. (2006); Ayla *et al.* (2005) and Ganesh and Aruna (1998). They reported that evaluation in the frequency of micronucleated polychromatic erythrocytes (MPCE) and micronucleated normochromatic erythrocytes (MNCE) increased significantly at 12, 24 and 36 h after treatment with lead nitrate at different doses.

On other hand, Schlick and Friedberg (1982) found that after oral administration of 1000 µg lead acetate/kg b.wt. for one month lead to a significant reduction of nucleated bone marrow cells.

Our previous data showed that intoxication with lead acetate lead to significant decrease in red blood cells count, Hb and PCV levels. These changes were accompanied by increased percentage of micronucleated polychromatic erythrocytes (MPCE). These results may suggest an disorder increased in erythropoiesis (Ayla, 2005 and Jagetial and Aruna, 1998).

In our study no significant difference had be shown between Gr 4 and Gr 1 in most of our data (Table 2, 4, 5, 7& 8) except total cholesterol, triglyceride and tissue residues (Table 3& 6). These parameters recorded significant increases when compared with control group. The increased concentration of total & free cholesterol

in serum after casein supplementation might be due to effect of casein on the enterohepatic cycle of bile acid. Casein increases the intestinal phosphate absorption, and decreases the faecal fat excretion (Roelof *et al.,* 1985).

Vahonuy, *et al.,* 1985 and George, *et al.,* 1985 reported that the increased total cholesterol and triglyceride in serum of rats fed a semipurified diet containing casein, due to the hyperlipidemia associated with decreased rates clearnance of chylomicro – like lipoprotein.

In the present study, there were significant improvement had been found in Gr 4 nearly in all our data (Table 2, 4, 5& 6)where, significant increase was found in lipid profiles in Gr 4 comparing with Gr 3, while there was significant increase in tissue residues between Gr 4 and Gr 3 (table 6). The improvement which was found in Gr 4 explained by Aisha *et al.,* 2009. Miller *et al*. (1970) and Springer 2005 who suggested that a possible relationship has been observed between susceptibility to lead toxicity and dietary content of protein. Theyfound that rats fed a protein free diet ratain twice as much lead as rats fed a 20% casein diet. In the same manner Hallen *et al,(*1996) recorded that lead is excreted into the milk bound to casein. The mechanism by which lead bond to casein was explained by Srinivas *et al*. (2007). He recorded that the protein (casein) combines with lead ions and minimizes its absorption by cross – linking free amino group and carboxylate group forming a precipitate. Bearnstein and Grand (1942) said that 20% casein in the diet protected rats from lead chloride (1.5%) whether the protective action of casein comes from reducing lead absorption or some other mechanism such as forming a less toxic lead – protein complex. Song *et al*. (1996) reported that hepatic Cu, Zn, SOD activity and hepatic GSH – PX concentration are higher in rats fed casein. The improvement was obviously clear in Gr 4 than Gr 3.

In the present study, slight significant difference had be shown between Gr 3 and Gr 4 in most our data (Table 2,3,4.5.6,7,8). These results were explained by Edrington *et al*. (1996) as they suggested that the efficiency of toxin binding activity considerably varies depending on the chemical structure of both the adsorbent and the toxin- exposure condition (pH, temperature) and duration of exposure. So that, this contradiction could be attributed to the dose of both toxin and adsorbent (AC) or nature of the toxin.

There were significant difference had be found between data in Gr 3 and Gr 2 in most of our data (Tablets 2,3 ,4,5,6).

Ademoyero (1983) found that administration of activated charcoal reduced GSH, selenium and cysteine; this reduction may due to reduce the toxic injury in liver. In addition Min and Young (2006) found that activated charcoal was decreased the increase of serum bilirubin and creatinine level induced by lead. So activated charcoal may protect from the lead induced toxicity on kidney.

Table 2: Some biochemical parameters in serum of control and treated rats with lead acetate and trails of treatment with casein or charcoal n=10

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)	GGT (U/L)	T. protein (g/dl)	T. bilirubin (mg%)
Gr1	48.52 ± 2.26^{a}	35.25 ± 1.8^{a}	22.14 ± 2.4^{a}	320.33 ± 17.69^{a}	48.15 ± 2.36^{a}	5.34 ± 0.3^{a}	0.9 ± 0.01^{a}
Gr2	64.72 ± 3.56^{b}	48.35 ± 2.6^{b}	34.42 ± 2.75^{b}	403.95 ± 20.3^{b}	58.32 ± 2.86^{b}	3.8 ± 0.2^{b}	1.93 ± 0.09^{b}
Gr3	56.32 ± 4.3^{c}	41.62 ± 1.83^{c}	28.15 ± 2.29^{c}	362.12 ± 18.3^{c}	54.9 ± 2.21^{c}	4.02 ± 0.32^{c}	1.21± 0.01^{c}
Gr4	49.91 ± 2.91^{a}	36.11 ± 2.03^{a}	21.35 ± 1.90^{a}	326.14 ± 17.05^{a}	47.01 ± 3.22^{a}	4.96 ± 0.39^{a}	0.98 ± 0.078^{a}
F-calculated	5.765#	23.765#	4.677#	9.654#	15.654#	11.456#	14.765#

Gr1 control
Gr2 treated with lead acetate 5%
Gr3 treated with lead acetate 5% + charcoal 0.05%
Gr4 treated with lead acetate 5% + casein 20%
Significant at $P < 0.05$ using ANOVA test
a, b insignificant difference between similar litter using Duncan Multiple Range test at $P < 0.05$

Table 3: Lipid profiles in serum of control and treated rats with lead acetate and trails of treatment with casein or charcoal n=10

group	T.cholesterol (µg/dl)	HDL (µg/dl)	VLDL (µg/dl)	LDL (µg/dl)	Triglycerides (µg/dl)
Gr1	90.4±2.49^{b}	26.5±0.52^{b}	19.3±0.62^{b}	44.6±3.9^{b}	82.26±3.11^{b}
Gr2	118.71±3.31^{a}	22.86±0.84^{a}	16.1±0.75^{a}	77.15±1.6^{a}	98.12±2.8^{a}
Gr3	96.84±3.47^{b}	25.3±1.12^{b}	18.95±0.77^{b}	52.65±4.15^{b}	79.13±3.42^{b}
Gr4	107.8±3.91^{c}	25.2±1.01^{b}	18.65±0.62^{b}	60.35±4.1^{b}	91.98±2.95^{a}
F-calculated	14.765#	9.876#	14.876#	8.965#	13.765#

Gr1 control
Gr2 treated with lead acetate 5%
Gr3 treated with lead acetate 5% + charcoal 0.05%
Gr4 treated with lead acetate 5% + casein 20%
Significant at $P < 0.05$ using ANOVA test
a, b insignificant difference between similar litter using Duncan Multiple Range test at $P < 0.05$.

Nagwa (2003) found that the treatment of rats with charcoal ameliorated adverse effects of toxin. Activated charcoal may be used as antioxidant and antidote in rats. The improvements were obvisonly clear in Gr (4) and Gr (3).

On the basis of the above findings it is concluded that, casein supplementation in combination with lead acetate minimized its marked alteration in some biochemical, antioxidant, essential elements, hematological and cytogenicity parameters.

Table 4: Serum level of zinc, iron, selenium, copper and calcium in control and treated rats with lead acetate and trails of treatment with casein or charcoal n=10

group	Zinc µg/dl	Iron µg/dl	selenium mg/dl	copper µg/dl	Calcium mg/dl
Gr1	15.61±0.71[a]	45.5±1.09[a]	8.12±0.45[a]	13.46±0.59[a]	9.2±0.5[a]
Gr2	11.75±0.82[b]	39.3±1.79[b]	5.2±0.32[b]	10.03±0.39[b]	5.5±0.49[b]
Gr3	13.4±0.46[c]	42.0±1.15[c]	6.5±0.37[c]	11.2±0.41[c]	7.23±0.45[c]
Gr4	14.8±0.58[a]	44.79±1.35[a]	7.96±0.52[a]	13.2±0.36[a]	8.9±0.64[a]
F-calculated	15.654#	14.652#	9.8769#	7.546#	8.546#

Gr1 control
Gr2 treated with lead acetate 5%
Gr3 treated with lead acetate 5% + charcal 0. 5%
Gr4 treated with lead acetate 5% + casein 20%
Significant at $P < 0.05$ using ANOVA test
a, b insignificant difference between similar litter using Duncan Multiple Range test at $P < 0.05$

Table 5: Antioxidant parameters in serum of control and treated rats with lead acetate and trails of treatment with casein or charcoal n=10

group	SOD(u/ml)	GSH-PX(u/ml)	CAT (u/ml)	GSH
Gr1	176.63 ± 5.67a	782.22 ± 73.82a	102.64 ± 5.51a	766 ± 31.7a
Gr2	127.8 ± 9.5b	520.12 ± 33.65b	137.56 ± 9.32b	574.3 ± 26.8b
Gr3	150.0± 12.45c	702.5 ± 41.8c	112.29 ± 7.07c	676.97 ± 40.3c
Gr4	170.3 ± 16.3a	778.6 ± 25.7a	103.64 ± 7.5a	720.2 ± 44.1a
F-calculated	12.65#	24,876#	9.876#	10.765#

Gr1 control
Gr2 treated with lead acetate 5%
Gr3 treated with lead acetate 5% + charcoal 0.05%
Gr4 treated with lead acetate 5% + casein 20%
Significant at $P < 0.05$ using ANOVA test
a, b insignificant difference between similar litter using Duncan Multiple Range test at $P < 0.05$

Table 6: Lead residue in serum and some tissues of control and treated rats with lead acetate and trails of treatment with casein or charcoal n=10

group	Serum µg/ml	Liver µg/g	Kidney µg/g	Muscle µg/g	Spleen µg/g	Intestine µg/g
Gr1	0.09±0.008[a]	0.06±0.003[a]	0.089±0.006[a]	0.042±0.008[a]	0.077±0.011[a]	0.028 ±0.002[a]
Gr2	0.92 ±0.07[c]	3.36±0.077[c]	16.6±0.011[c]	1.3±0.04[d]	0.51±0.008[d]	1.102 ±0.018[c]
Gr3	0.23 ±0.02[b]	0.93±0.008[b]	6.44±0.007[b]	0.96±0.015[c]	0.28±0.006[c]	0.523 ±0.007[b]
Gr4	0.21 ±0.02[b]	0.693±0.05[b]	0.055±0.004[a]	0.305±0.004[b]	0.166±0.011[b]	0.51 ±0.006[b]

Gr1 control
Gr2 treated with lead acetate 5%
Gr3 treated with lead acetate 5% + charcoal 0.05%
Gr4 treated with lead acetate 5% + casein 20%
Significant at $P < 0.05$ using ANOVA test.
a, b insignificant difference between similar litter using Duncan Multiple Range test at $P < 0.05$

Table 7: Hemogram in serum of control and treated rats with lead acetate and trails of treatment with casein or charcoal n=10

group	RBCs($x10^6$/ul)	Hb(g/dl)	PCV(%)
Gr1	5.44 ± 0.37a	18.73 ± 0.627a	43.5 ± 1.14a
Gr2	3.11 ± 0.28b	14.3 ± 0.72b	36.05 ± 0.95b
Gr3	4.03 ± 0.3c	16.5± 0.51c	39.34 ± 1.19c
Gr4	5.02 ± 0.49a	17.96 ± 0.48b	42.85 ± 1.29a
F-calculated	9.965#	10.654#	11.543#

Gr1 control
Gr2 treated with lead acetate 5%
Gr3 treated with lead acetate 5% + charcoal 0.05%
Gr4 treated with lead acetate 5% + casein 20%
Significant at $P < 0.05$ using ANOVA test
a, b insignificant difference between similar litter using Duncan Multiple Range test at $P < 0.05$

Table 8: Incidences of MPCE on the of PCE to NCE in bone marrow in control treated rats with lead acetate (0.5g / 100 ml water)with trails of treatment with charcoal or casein n=10

Group	PCE	MPCE	CPMCE	%	NCS	PCE/NCE
Gr1	5000	28	5.6 ± 0.32[a]	0.56	2183	2.29 ±0.19[a]
Gr2	5000	41	7.6 ±0.05[b]	0.76	1295	3.86 ±0.32[b]
Gr3	5000	30	4.3 ±0.42[a]	0.43	1996	2.1 ±0.12[a]
Gr4	5000	27	5.1 ±0.22[a]	0.51	2075	2.41 ±0.15[a]
F-calculated	-	-	9.876#	-	-	16.876#

Gr1 control
Gr2 treated with lead acetate 5%
Gr3 treated with lead acetate 5% + charcoal o. 5%
Gr4 treated with lead acetate 5% + casein 20%
Significant at $P < 0.05$ using ANOVA test
a, b insignificant difference between similar litter using Duncan Multiple Range test at $P < 0.05$

REFERENCES

Adebayo, O.L., B.O. Adegbesan and G.A. Adenuga, 2009. Comparison of the effects of low protein diet versus chemical neurotoxins on brain weight, brain lipid per oxidation and antioxidant status of rats .Asian Journal of Biological Sciences, 2(1): 7-13.

Ademoyero, A. and R.R. Dalvi, 1983. Efficacy of activated charcoal and other agents in the reduction of reptotoxic effects of asiangle dose of aflatoxin B1 in chickens. Toxicology lethersi 16(1and 2): 153-157.

Ademuyiwa, O., N.U. Regina, I. Florence and A. Olugbenga, 2005. Plasma lipid profiles and risk of cardiovascular disease in occupational lead exposure in Abeokuta Nigeria – lipids in Heath and Disease; 4: 19.

Ahamed, M. and M.K.J. Siddiqui, 2007. Low level lead exposure and oxidative stress: current opinions. Clin. Chim. Acta., 388: 57-64.

Ahmed, Y.F. and S.I.A. Shalaby, 1999. Clincopathological and histopathological studies on chronic lead intoxicated in male Bakri sheep. Afric. J. Agri Sci., 18: 19-37.

Aisha, M. Fahim; Z. Maha, Elabedein Gaber and Ansam, M. Salman, 2009. Protective effects of casein or activated charcoal on lead toxicity upon fertility and immunological state in male rats. Egypt J. Biomed. Sci., 31: 316- 331.

Al. Bekairi, A.M., S. Qureshi, M.A. Chaudhryand A.H. Smh, 1991. Uric acids inhibitor of cyclophosphamide – induced micronuclei in mice. Mut. Res., 262: 115-118.

Alghazal, M.A., I. Sutiakova, N. Kovalkovicova, Legath, M. Ji Falis, Pistt, Ji Sabo, K. Ri Benova, Sabova, Li and P. Vaczi, 2008. The induction of micronuclei in rat bone marrow after chronic exposure to lead acetate trihychate. Journal toxicology and industrial health, 24(9): 587-593.

Alvares, A.P., A. Fischbein, S. Sassa, K.E. Anderson and Kappas, 1976. Lead intoxication : effects on cytochrome P- 450 – mediated hepatic oxidations. Clin. Pharmacol. Ther., 19: 183-190.

Alvares, A.P., S. Kapelner, S. Sassa and A. Kappas, 1975. Drug metabolism in normal children, lead poisoned children and normal adults. Clin. Pharmacol. Ther, 17: 179-183.

Aly, M.H., H. Kim, S. Renner, A. Boyarsky, M. Kosmin and D.E. Paglia, 1993. Hemolytic amemia associated with lead poisoning from shotgu pellets and the response to succimer treatment. American Journal of Hematology, 44: 280-283.

Anetor, J., T.S. Akingbola, F.A. Adeniyi and Gotylor, 2005. Decreased total and ionized calcium levels and haematological indices in occupational lead exposures evidence of the endocrine disruptive effect of lead. Indian journal of Occupational & Environmental Medicine, 9(1): 15-21.

Anetor, J.I., 2002. The probable situation of endocrine disruption in the highly polluted Nigerian environment. Yokohama, Japan. Book of collective abstract and programme, pp: 67-88.

Antinio, M.T., L. Corredor and M.L. leret, 2003. Study of the toxicity of several brain enzymes like markers of neurotoxicity induced by prenatal exposure to lead and / or cadmium. Toxicol. Let., 143: 331-340.

Anurdha, M., 2007. Lead hepatotoxicity & potertial heath effects. Indian J. Med. Res. 126 Decemer, pp: 518-527.

Ariss, J.D., S. Vinogradov and B. Zak, 1981. "Clin. Biochem"; 14: 311-315.

Asa, G., H. Pavo, S. Andrejs and S. Staffan, 1989. Occupational lead exposure and pituitary function International archives of occupational and environmental heath, 61(4): 277-281.

Assmann, G., 1979. Current diagnosis of hyperlipedemias. Internist. (Berl.), 20: 559-664.

Ayla, Ç., O. Oya and Ç. Ülkü, 2005. The evaluation of micronucleus freguency by acridine orange fluorescent staining in peripheral blood of rats treated with lead acetate . Mutagenesis, 20(6): 411-415.

Azab M. S. and Peterson, P. J. (1999): The removal of cd from waste water by the use of biological sorbent water science. Techni. 21 : 1705 – 1706.

Baars, A. J.; Spierenburg, I. J. And Graaf, G. J. (1988): Chrome lead and cadmium poisoning in farm animals. 2nd annual conference on trace. Substances in environ. Health. St. touis, Missouri 23 – 25 May (1988) Edited by Hemophill, D.D.

Bachorik, P. S. and Ross, J. W. (1995): National cholesterol education program recommendutions for measurement of low density lipo protein cholesterol; Executive summary. Clinical chemistry; 41(10): 1414 – 1420.

Baernstein, H. D. and Grand, S. A. (1942): The relation of protein intake to lead poisoning in rats. J. Pharmacol. Exp. Ther. 74: 18.

Bernard, F.; Jose Ph, G. and Nemi, C. (2000): Schalm's veterinary Hematology 5th edlippino citt Williams and Wilkins co. Philadephia, Baltimor.New York, London, HongKong, Tokyo.

Bettiger, W. J.; Reeves, P. G.; Savage, J. E. and O'dell. B. L. (1980): Interaction of zinc and vitamin E in the chick. Proceedings of the society for experimental Biology and Medicine; 163 : 432 – 436.

Beuter, E. (1982): In Red cell metabolism, a manual of biochemical method. E Beuller (eds), Grune and Stratton, New York PP 105 – 106

Beutler, E.; Gelbart T. (1969): Improved assay of the enzyme of glutathione syntheses gamma glutamylayst synthetase and glutathione synthetase. Clin. Chim. Acta; 158: 115 – 123.

Blanusa, M.; Piasek, M. and Kostial, K. (1989): Interaction of lead with some essential elements in rat's kidney in relation to age. Biol. Trace Elem. Res.; 21: 189 – 93

Blaxter K. L. 1950. Lead as nutritional hazard to form livestock. 111. factors in fluencing the distribution of lead in the tissues. J. Comp. Pathol. Ther. 60: 177.

Bogden, J. D.; Kemp, F. W.; Han, S.; Murphy, M.; Fraiman, M.; C Zerniach, D.; Flynn, C. J.; Banna, M. L.; Scimone, A.; Castrovilly, L. and Gertner, S. B. (1995): Dietary calcium and lead intract to modify material blood pressure. Erythro poiesis and fetal and neonatal growth in rats during pregnancy and lactation. J. Nutrition; 125: 990 – 1002.

Chajet, S. T, Friedman, G.; Stein, O.; Shiloni, F.; Etienne, J. and Stein, Y. (1989): Mechanism of the hyperteriglyceridemia induce by tumor necrosis factor administration to rats. Biochim. Biophys. Acta; 1001: 316 – 324.

Cupic, V.; Dobric, S.; Milovanovic, Z. and Bokonjic (2003): The efficacy of activated charcoal and klinoptiolite in protection of animals poisoned by bromadiolone. Toxic. Let.; Vol. 144, P. 175.

De Silva, P.E. (1981): Determination of lead in plasma and studies on its relationship to lead in erythrocytes .British Journal of Tndustrial medicine, 38:209-217.

Dhir, H.; Kumar, A.; Sharma, A.; Talukder, G. (1990): Modification of clastogenicity of lead and aluminum in mouse bone marrow cell by dietary in gestion of phyllanthus emblica fruit extract mutation research, 241, 305 – 312.

Dominiczak,. M. H. and Mc Namara, J. R. (2000): The system of cardiovascular prevention. In Handbook of lipoprotein testiny. Eds., Rifai N., G. R. warnick and M. H. Dominiczak. American Association for clinical chemistry Inc, Washington, PP: 103

Doyle, J. J. and Younger, R. L. (1984): Influence of ingested led on distribution of lead, iron, zinc, copper and manganese in bovine tissues. Vet. Human. Toxicol.; 26: 201 – 204.

Dumas, B. T.; Watson, W. and Biggs, H. G. (1971): Direct colorimetric method for determination of serum albumin. Clin. Chem. Acta. 31: 87.

Durgut, R. Koc, A. Gonenci, R. Bal, R. Celik, S. Guzaf, M. Altug,M.E. and Atesoglu ,O.(2008):Effect of high dose lead toxication on liver ,kidney , heart, brain and blood rabbits: an experimental study . J. Appl. Biol. SC. 2 (2) : 11-18.

Edrington, T. S.; Sarn, A. B.; Kubena, L. F.; Harvery, R. B. and Philips, T. D. (1996): Effects of hydrated sodium calcium aluminosilicate (HSCAS), acidic HSCAS and activated charcoal on metabolic profile and toxicity of aflatoxin B_1 in turkey poults. Toxic. Let.; 89: 115-122.

Ellenhorn (1997): Medical toxicology Zn ed. William and Wilkins.

Estela Kristal – Boneh, Doron coller, Danl froom, Gil Harari and Tose Ph Ribak (2005): The Association between occupational lead exposure and serum cholesterol and lipoprotein levels. American Journal of Public Health; July vol. 89 No. 7.

Evans, P. and Halliwell, B. (2001): Micronutrients. Oxidant / antioxidant status. British J. Nutr.; 855:57 – 74.

Fahim, A.M.; Zaki,A.A.; and Ibrahim, A M.(2009): Protective effects of vitamin C and zinc against lead toxicity and immunization with rodococcus equi antigen on some biochemical and immunological parameters in male rats. Egypt J .Basic Physiol., 8(2) :391-414.

Farmand, F.; Ehdai, A.; Roberts, C. K. and Sindhu, R. K. (2005): lead – induced dyseregulation of superoxide dismutases, catalase, glutathione peroxidase, and efuanylate cyclase. Environ. Res.; 98: 33 – 39.

Fatma, M. Fakhry, 1992. Teratogenecity and placental transfer study of lead in relation to LD_{50} in albino rats. The new Egyptian J. of Medicine, 3(6): 1220-1229.

Fauci, A., E. Braunwald, D. Kasper, S. Hanser, D. Longo, J. Jameson and Loscalzo, 2008. Harrison's Principles of internal Medicine. Mc Graw – Hill professional, united States. Retrieved January, 252010, in http://www.medicinenet.com

Flora, S.J., V.K. Jain, J.R. Behari and S.K. Tandon, 1982. Protective role of trace metals in lead intoxication. Toxicol. Lett., 13 P 52.

Frangenberg, J., 1986. Pb, Cd, Fe and Cu in organen Von Krankten Kalbern Zur erlangung des doctor grades beim fachbereich vetrinar medizin lustus liebig. Universtate Gie Ben.

Ganesh, G.J. and R. Aruna, 1998. Effect of various concentration of lead nitrate on the induction of micronuclei in mouse bone marrow . Mutat. Res., 415: 131-137.

George, V.V., I. Adamson, W. Chalcarz, S. Satchithanandam, R. Muesing, D.M. Klurfeld, S.A. Tepper, A. Sanghvi and David, Kritchevsky, 1985. Effect of casein and soy protein on hepatic and serum lipids and lipoprotein lipid distributions in the rat. Journal of Clinical lipidology, 56(2): 127-137.

Ghayour- Mobarhan, M., A. Taylor, S.A. New, D.J. Lamb and G.A. Ferns, 2005. Determinants of serum copper , zinc and selenium in healthy subjects. Ann. Clin. Biochem., 42: 364-375.

Glinder, E.M. and T.D. King, 1972. Rapidcolorimetric determination of calciuminbiological fluids with methyline blue. Am. J. Clin. Path., 58: 376-382.

Goering, P.L., 1993. Lead – protein interactions as a basis a basis for lead toxicity. Neurotoxicol., 14: 45-60.

Gontzeer, I., P. Gutzesco, D. Corona and D. Lungu, 1964. Lead toxicity. Arch. Sci. Physiol., 18: 211- 224.

Graig, D.S. and C. Wayne, 1984. Simple automated wet digestion of animal tissue for determination of sever element by atomic absorption spectroscopy. Technical Communication., 107: 115.

Gurer, H.M.G., R. Ozgunes, D.R. Neal, N. Spilaz and Ercal, 1998. Antioxidant effects of N – acetyle cystein and succimer in red blood cells from lead exposed rats. Toxicol., 128: 181- 189.

Hallen, I.P., S. Jonsson, M.D. Karlsson and A. Oskarsson, 1996. Kinetic observation in neonatal mice exposed to lead via milk. Toxicol. Applied Pharmacol., 140: 13-18.

Hamadouche, N., M. Slimani and A.E.K. Aous, 2009. Biochemical parameters alterations induced by chronic oral administration of lead acetate in albino rat. Am. J. Sci. Res., 4: 5-16.

Hami, J., R. Dashti Gh Nemat, M. Bakhsh, M. Afshar and H.R. Ghaffari, 2006. The relationship between high dose lead exposure and serum lipids and lipoprotein levels. Shiraz E- Medical Journal, 7: 2 April.

Hande, G. and E. Nuran, 2000. Can antioxidants be beneficial in the treatment of lead poisoning. Free radical biology & Medicine, 29(10): 927-945.

Hart, J.W. and H. Engberg – Pederson, 1983. Statistics of the mouse bone marrow micronucleus test counting distribution and evaluation of results. Mutat. Be., 111: 195-207.

Hartwig, A., R. Schlepegrell and D. Beyersmann, 1990. Indirect mechanism of lead induced genotoxicity in cultured mammalian cells . Mutat. Res., 241: 75-82.

Herman, D.S., M. Geraldine and T. venkatesh, 2009. Influence of minerals on lead – induced alterations in liver function in rats exposed to long term lead exposure J. Hazard Mate., 166(2–3): 1410-1414.

Jaffe, E.K., J. Martins, J. Li, J. Kervinen, And R.L. Dunbrack, 2001. The molecular mechanism of lead inhibition of human porphobilinogen synthase. J. Biol. Chem., 276-1531.

Jaffe, E.K., M. Volin, C. Bronson-Multins, R.L. Dunbrack, J. Kervinen, J. Martins, J.F. Quinlan, M.H. Sazinsky, E.M. Steinhouse and A.T. Yeung, 2000. An artificial gene for human porphobilinogen sythase allows comparison of an allelic variation Implicated in susceptibility to lead poisoning . J. Biol> Chem. 275: 2619.

Jin, C., Y. Li, Y. Li, L.Y. Zou, G.L. Zhang, M. Normura and G.Y. Zhu, 2008. Blood lead : Its effect on trace element levels and iron structure in hemoglobin nuclear instruments and methods in physics research B 266: 3607-3613.

Jindal, N., S.K. Mahipal And N.K. Mahajan, 1994. Toxicity of aflatoxin B_1 in broiler chicks and its reduction by activated charcoal. Res. Vet. Sci., 56(1): 37-40.

Khan, M.S.H., M. Mostafa, M.S. Jahan, M.A. Sayed and M.A. Hossain, 2008. Effect of garlic and vitamine B. complex in lead acetate induced toxicities in mice Bangl. J. Vet. Med., 6(3): 203-210.

Kulikowska, K.E. and J.J. Moniuszko, 2001. Lead and zinc influence on antioxidant enzyme activity and malondialdehyde concentrations. Polish. Journal of Environmental Studies., 10(30): 161-165.

Linda, S., Tuffl, Cheryl F. Nockels and Martin, Fettman, 1988. Effect of E. coli on iron copper and zinc metabolism in chicks.

Lubran, M.M., 1980. Lead toxicity and heme biosynthesis, Annal. Clin. Lab. Sci., 10: 402.

Lynda Allouche, Mohamed Hamadouche, Abderrezek Touabti and Seddik Khennouf, 2011. Effect of long-term exposure to low or moderate lead concentrations on growth lipid profile and liver function in albino rats. Advances in biological research, 5(6): 339-347.

Majkic- Singh, N., 1993. Klinicka enzinmologija. Univerzitet U Beogvadu: 239- 324.

Makino, T., M. Saito, D. Horiguchi and K. Kina, 1982. "Clin Chim " Acta, 120: 127-135.

Mehana, E.E., A.M.A. Meki, And K.M. Fazili, 2010. Ameliorated effects of green tea extract on lead induced liver toxicity in rats. Exp. Toxicol. Pathol. Doi: 10: 1016 / J. etp

Miller, J.A., V. Batlistini, R.L.C. Cumming, F. Carswell and A. Goldberg, 1970. Effect of lead on mammalian reproduction. Lancet, 2: 695.

Milnes, M.R., D.S. Bermudes, T.A. Bryan, T.M. Edwards, M.P. Gunderson, T.L. Larkin, B.C. Moore and L.J. Guillette, 2006. Contaminant induced feminization and demasculinization of non mammalian vertebrate male in aquatic environments. Environ. Res., 100: 3-17.

Milton, R.H., B. Earl, B.B. Dawson and J. Williams, 1982. Tissue distribution of lead in rat pups nourished by lead poisoned mothers. J. Toxicol. Environ. Heath, 9: 7786.

Min, J.C. and B.R. Young, 2006. Protective effects of activated charcoal on the acute damages of kidney of mouse by lead . Korean J.Electron Microscopy, 36(2): 57-72.

Mirsa, H.P. and Fridovich, 1972. The role of superoxide anino in the anto oxidation of epinephrine and simple assay for superoxide dismutase J. Biol. Chem., 247: 3170-3175

Missoun, F., M. Slimani and A. Aoues, 2010. Toxic effect of lead on kidney function in rat wistar. African Journal of Biochemistry Research, 4(2): 21- 27.

Moussa, S.A. and S.A. Bashandy, 2008. Biophysical and biochemical changes in the blood of rats exposed to lead toxicity. Romanian. J. Biophy, 18: 123-133.

Nabil, M. Ibrabim; Esam, A. Eweis; Hossams, El-Beltagi and Yasmin, E. Abdel – Mobdy, 2012. Effect of lead acetate toxicity on experimental male albino rate. Asian Pacific Journal of Tropical Biomedicine; 41-46.

Nagwa, M. El- Sawi Mahmoud, 2003. Effect of activated charcoal on trichoverins (A&B) toxin administration on rat liver and serum protein. J. App. Anim. Res., 23: 175-184.

Nashwa, A. Abu-Aita, S. Sherein, Abd El-Gaied, and Mogda, K. Mansour, 2006. "Clinicopathological studies on the ameliorative effect on *Nigella sativa* oil against genotoxicity and oxidative damage in lead intoxicated rats." J. Egypt. Vet. Med. Assoc., 66(4): 127-146.

National Academy of sciences, 1972. Lead air borne lead in perspective Commette on biologic effects of atmospheric pollutants. Division of Medical sciences National Academy of sciences. Washington Dc.

Neathery, M.W. and W.J. Miller, 1975. Metabolism and toxicity of cadmium, mercury and lead in animals. Review. J. Dairgsci., 58: 1767-1780.

Noriega, G.O., M.L. Tomaro and A.M. Del Battle, 2003. Bilirubin is highly effective in preventing in vivo delta – aminolevulinic acid – induced oxidative cell damage. Biochem. Biophys. Acta, 1638(2): 173-178.

Paton, G., 1973. effect of certain metal ions on DNA repair in mammalian cells. Mut. Res., 21: 199-201.

Patora, R.C. and D. Swarup, 2004. Effect of antioxidant ascorbic acid, 1 – methionine or & tocopherol alone or along with chelator on cardiac tissue of lead – treated rats. Veterinarhiv, 74(3): 235-244.

Patrick, L., 2006. Lead toxicity part Π the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity, Altern. Med. Rev., 11: 114.

Reitman, S. and S. Frankel, 1957. A colorimetric determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Path., 28: 56.

Ribadeaudumas, A.R., 1972. Eur. J. Biochem., 25: 505

Richmond, W., 1973. Acolorimdric determination of serum cholesterol. Clin. Chrm., 19: 1350-1356.

Roelof van der Meer, Hielke De Vries, clive E. West and Hugo De Waard, 1985. Casein – induced hypercholesterolaemia in rabbits is calcium dependent. Atherosclerosis., 56(2): 127-156.

Ryan, H. and M.W. Aldoori, 2005. The relevance of selenium to immunity. Cancer and infectious/inflammatory diseases. Can J. Diet. Pract Res., 66: 98.

Salamon, M., J. Heddle, E. Stuart and M. Katz, 1980. Towards an improved micronucleus test. Studies on 3 model agents, mitomycine, cycolophosphamred and dimethyl benzanthracene. Mutant. Res., 74: 347-356.

Salma, K. and D. Kshama, 2005. Effect of withania somnifera root extract on lead – induced DNA damage. Journal of food, Agriculture and Environment., 3(1): 31-33.

Schlich, E. and D. Friedberg, 1982. Bone marrow cells of mice under influence of low lead doses . Arch .Toxicol., 49: 227-236.

Seddik, L., T.M. Bah, A. Aoues, M. Brnderdour and M. Silman, 2010. Dried leaf extract protects against lead – induced neurotoxicity in wistar rats. Eur. J. Sci. Res., 42(1): 139-151.

Shalan, M.G., M.S. Mostafa, M.M. Hassouna, S.E. Hassab El – Nabi and A. El – Rafaie, 2005. A melioration of lead toxicity on rat liver with vitamin C and silymarin supplements. Toxicology, 206: 1-25.

Simons, T.J.B., 1995. The affinity of human Gythrocyte porphobilinogen synthase for Zn^{2+} and Pb^{2+}. Eur. J. Biochem., 234:178.

Snedecore, G.W. and W.G. Cochran, 1980. Statistical methods. 6th Erd., Lowa stateuniv. Press Ames, Lowa USA.

Song, Y., S. Igawa and A. Horii, 1996. Antioxidant enzymes response to endurance exercise training and dietary proteins in rat skeletal muscle and liver. Appl. Human Sci., 15(5): 219-225.

Spomenka, T., C. Bozo, P. Alica, J. Jasna and C. Peter, 2007. Reproductive toxicity of low – level lead exposure in men. Environmental research, 10: 256-266.

Springer, N., 2005. Influence of dietary protein composition on lead absorption in rats. Bulletin Environmental Contamination and Toxicology (may): 1- 127.

Srinivas, S., P. Kaul and V. Prakash, 2007. Mechanism of interaction of Bp with milk proteins a case study of alph-casein. J. Agric food chem., 31(22): 928.

Sujatha, K., C.H. Srilatha, Y. Anjaneyulu and P. Amaravathi, 2011. Lead acetate induced nephrotoxicity in wistar albino rats. A pathological immunohistochemical and ultrasructural studies. International Journal of Pharma. and Bio. Sciences.

Szasz, G and J.P. Persjn, 1974. Acolorimdric determination of serum LDH .Clin. Biochem., 12: 228.

Tamura, M., N. Oschino and B. Chance, 1982. some characteristics of hydrogen and alkylhy dho peroxides metabolizing system in cardiac tissues J. Biochem., 92: 1019-1031.

Tarugi, P., S. Calandra, P. Borella and G.F. vicvoli, 1982. Effect of lead intoxication on rabbit plasma lipoproteins S. Atherosclerosis., 45: 221- 234.

Todd, A.C., J.G. Wetmur, J.M. Moline, J.H. Godbold, S.M. Levin and P.J. Landrigan, 1996. Unraveling the chronic toxicity of lead : an essential priority for environmental health, environ. Health Perspect., 104-141.

Todorovic Tatjana, Dozic I, Vujanovic Dragana, J. Pejovic and M. Marjanovic, 2005. The influence of chronic lead poisoning on the activity of some serum enzymes in rats Acta Veterinaria (Beograd), 6: 471-482.

Toxicol., 128: 181-182.

Upasani, C.R. and R. Balaraman, 2001. Effect of vitamin E, vitamin C and spirulina on the levels of membrane bound enzymes and lipids in some organs of rats exposed to lead Indian Journal of pharmacology, 33: 185-191.

Vahouny, G.V., J. Adamson, W. Chalcarz, S. Satchithanandam, R. Muesing, D.M. Klurfeld, S.A. Tepper, A. Sanghvi and D. Kritchevsky, 1985. Effects of caseim and soy protein on hepatic and serum lipids and lipoprotein lipid distributions in the rat. Atherosclevrosis Aug: 56(2): 127-137.

Valverde, M., T.I. Fortoul, F. Diaz-Barriga, J. Mejia and O.E. Rojas Delcastill, 2002. Genotoxicity induced in C D-1 mice By Inhaled lead : differentionl organ response. Mutagenesis., 17: 55-61.

Veena, S., S. Saidhana, Pracheta, P. Ritu, and S. Shatruham, 2011. Therapeutic efficacy of withania somnifera root extract in the regulation of lead nitrate induced nephrotoxicity in swiss albino mice. Journal of pharmacy Research, 4(3): 755-758.

Wahlrfeld, A.W., 1974. In methods of enzymatic analysis, Vol. 5, Huberymeyer, Ed. Academic press, New York, pp: 1831-1835.

Walter, M. and H. Gerade, 1970. ultra micro method for determinateion of conjugated and total bilinbim in serum or plasm Microchem. J. 15(2): 231-243.

Warnic, G.R. and J.J. Albers, 1978. Comprehensive evaluation of the reparin manganese precipitation procedure for estimates HDL cholesterol. J. Lipid Res., 19: 65-67.

William, H., 1980. official method of analysis of the association of official analytical chemists thirteen edition Washington DC USA

Winston, W.K., K. Susan Kwispe, J. Jean Steichen, C. Reginald Tsang, Paul. A. Succop, Robert L. Born Schein, Omer G. Bergen, 1991. Serum vitamin D metabolites and bone mineralization in young children with chronic low to moderate lead exposure – pediatrics, 87(5): 680 -687.